

## Clinical and laboratory aspect of leptospirosis in humans

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### ABSTRACT

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Leptospirosis is a world-wide zoonotic disease, especially in tropical and subtropical regions. The disease is found in rural and urban areas with poor environmental conditions. The spectrum of human disease ranges from subclinical infection to severe clinical disease with multi-organ failure (Weil's disease) and high mortality rate, and depends both on the host and the infecting serovar. Leptospirosis may occur either sporadically or in the context of an outbreak and is commonly related to occupational or recreational activities that involve direct or indirect contact with the urine of animal species that are reservoirs of the disease. The disease infects man through contact with contaminated environments or direct contact with carrier animals such as rats, dogs, cattle, pigs, etc. Leptospirosis has clinical symptoms similar to such diseases as dengue, malaria, typhoid, influenza, thus laboratory methods are required for early detection to facilitate appropriate treatment of patients. The diagnosis of leptospirosis should be considered in any patient presenting with an abrupt onset of fever, chills, conjunctival suffusion, headache, myalgia, and jaundice. Suspicion is further increased if there is a history of occupational or recreational exposure to infected animals or to an environment potentially contaminated with animal urine. Treatment commonly consists of administration of antibiotics such as penicillin, ampicillin, amoxicillin, tetracycline and doxycycline. Prevention by immunization is uncommon, as the available vaccines are ineffective in preventing the disease. In general, prevention is by avoiding environmental exposure.

**Keywords:** Leptospirosis, clinical symptoms, laboratory, treatment

### INTRODUCTION

Leptospirosis in humans is caused by *Leptospira* bacteria transmitted through bites of infected animals, contaminated water and soil, or through occupations in the dairy industry and agriculture.<sup>(1,2)</sup> There have been reports of

*Leptospira* infection in tourists after travelling and in sportsmen after participating in matches in areas endemic for leptospirosis.<sup>(3)</sup> The clinical manifestations of the disease are extremely variable. The majority of patients (90%) show mild symptoms similar to those of influenza, while the rest have very severe symptoms

consisting of hepatic, renal, pulmonary and central nervous system abnormalities.<sup>(4,5)</sup>

The clinical symptoms of leptospirosis are similar to those in other diseases, such as hepatitis, influenza, dengue hemorrhagic fever, and typhoid fever,<sup>(2,5)</sup> so that it is extremely difficult to establish a definite diagnosis of leptospirosis. To date, the most reliable diagnostic test is the microagglutination test (MAT). However, this test is extremely complicated, because it requires expert personnel and a large number of live serovars of the bacterium. Therefore a search for alternative tests is mandatory. The treatment of this disease is actually extremely simple, with the administration of penicillin G, doxycycline, ampicillin, or amoxicillin. The prognosis is relatively good, but in severe cases the mortality rate is around 5-52%.<sup>(4,6)</sup> *Leptospira* was first discovered simultaneously in Japan and Germany from autopsy specimens of patients diagnosed as having had yellow fever.<sup>(4)</sup> The problem of differentiating jaundice due to leptospirosis and yellow fever continued to exist until the deaths of Stokes and Noguchi while investigating the causative organism.<sup>(4)</sup>

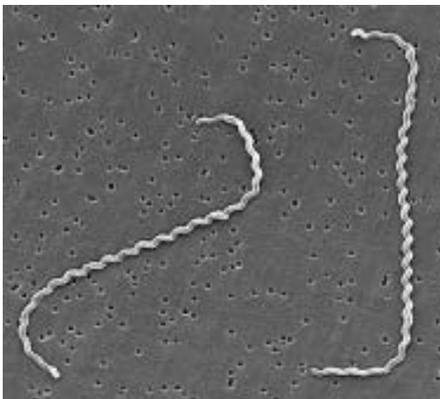


Figure 1. Electron micrograph of *Leptospira interrogans* fixed on a 0.2  $\mu$ m filter<sup>(10)</sup>

## Epidemiology

Leptospirosis is endemic throughout the world. The majority of infections in the tropics are mainly encountered in the rainy season,<sup>(5,7)</sup> while in the subtropics the disease is mostly found in summer. Epidemics usually occur in seasons with a heavy rainfall.<sup>(8)</sup> In nature *Leptospira* is found in carrier animals suffering from chronic renal disease, so that during the entire lifespan of the animals their urine continuously contains *Leptospira* organisms. Infection usually occurs through direct or indirect contact with infected animal urine or tissues.<sup>(4)</sup> The most important animals as a source of transmission are rats, followed by dogs, cattle and pigs.<sup>(9)</sup> The disease is commonly transmitted indirectly as an occupational hazard, such as in abattoir workers, meat vendors, workers in the fruit and meat canning industries, and in farmers.<sup>(2)</sup> There have been reports of swimming pools contaminated with *Leptospira*, so that a large number of swimmers were affected with leptospirosis.<sup>(1)</sup> In the tropics, individuals most frequently infected are farmers in contact with wet soils contaminated with *Leptospira*-containing urine, e.g. rice and potato farmers. It has also been reported from Israel that troops stationed near the Jordan river have been infected with *Leptospira*. Lastly the presence of leptospirosis has been reported in tourists travelling in areas endemic for *Leptospira*.<sup>(3,4)</sup>

## Microbiology

Leptospire are tightly coiled spirochetes, usually 0.1 mm by 6 to 0.1 by 20 mm, but occasional cultures may contain much longer cells. The helical amplitude is approximately 0.1 to 0.15 mm, and the wavelength is approximately 0.5 mm. The cells have pointed ends, either or both of which are usually bent into a distinctive hook (Figure 1).<sup>(10)</sup>

On the basis of genetic homology, there are currently more than one recognized species,

which proves the existence of an extremely variable expression of the *Leptospira* genome. Based on a taxonomy currently being developed, *Leptospira* is divided into 12 species: *L. alexanderi*, *L. biflexa*, *L. borgpetersenii*, *L. fainei*, *L. inadai*, *L. interrogans*, *L. kirschneri*, *L. Noguchi*, *L. santarosai*, *L. weilii*, *L. meyeri*, and *L. wolbachii*. All known species are divided into pathogens, intermediates, and saprophytes. The terms *L. interrogans* and *L. biflexa* have traditionally been used for distinguishing pathogenic and saprophytic strains.

### Pathogenesis

Although the pathology due to leptospirosis has been known for a long time, not much is known about its pathogenesis. *Leptospira* invades the body through abrasions in the skin, mucous membranes, and conjunctiva. The bacteria may also enter through inhalation of macroscopic aerosol droplets. Transmission through food is unimportant, but may allow *Leptospira* to enter by way of the mucous membranes. *Leptospira* entering the blood may spread to all organs of the body.<sup>(4)</sup> The variety of clinical manifestations seen in patients is due to extensive vasculitis throughout the body. The symptoms occurring are extremely variable, with an onset from one day up to four weeks after exposure. Recovery may take more than two months, or even more than six months.<sup>(11)</sup>

There are two phases in the pathogenesis of leptospirosis: the leptospiremic phase and the immune phase. As mentioned previously, *Leptospira* enters the human body through abrasions on the surface of the skin, mucous membranes, and conjunctiva. Subsequently the organisms enter the circulation, undergo replication and are carried to various organs in the body, thus causing extensive clinical symptoms. Infection through the respiratory and gastrointestinal tracts is considered insignificant.<sup>(12)</sup>

Hepatic damage takes the form of disorganization rather than injury to hepatocytes. *Leptospira* invades and damages the hepatic sinusoids, spaces of Disse, and parenchymal cells. The bacterium may also enter the spaces between the parenchymic cells and damage the canaliculi, thus causing bilirubin overflow into the systemic circulation and subsequent hyperbilirubinemia, generally without an accompanying increase in hepatic enzymes. There is also disorganization or blockage of the sinusoids, while the Kupffer cells are commonly swollen, containing *Leptospira* organisms and red blood cells. In the liver there are also focal hemorrhages, petechiae and interstitial edema.<sup>(4,11)</sup>

The pathologic findings encountered in the kidneys are due to hypoxia. In addition there are lesions in the kidneys appearing concurrently with impairment of renal function, thus being suggestive of intracellular damage due to toxins. There is also vasculitis and hemorrhage accompanied by interstitial edema, necrosis of the tubular epithelium, and damage to the basal membrane.<sup>(11)</sup> In severe cases, the kidneys are swollen and yellowish, particularly in the [areas around the] cortical vessels. Histologically, in the tubulointerstitial areas there is diffuse infiltration of lymphocytes, plasma cells and polymorphs, accompanied by localized areas of tubular necrosis.<sup>(4)</sup> The renal damage was subsequently demonstrated by the presence of abnormal urinalysis findings, i.e. proteinuria, sterile pyuria, hematuria, along with hyaline and granular casts. Serum urea and creatinine levels also tended to be elevated.<sup>(12)</sup>

*Leptospira* may also attack the lungs, leading to hemorrhage, dyspnea, hemoptysis, and respiratory failure.<sup>(5)</sup> A great number of cases have died from respiratory failure, without renal and hepatic abnormalities; no inflammatory processes were found in the lungs of these patients.<sup>(8)</sup> Hemorrhages were found throughout

the respiratory system, including the trachea, alveoli, and interstitium. The pulmonary vessels showed endothelial injury, and leucocytic and platelet thrombi, thus suggesting infarction. The occurrence of hypoxia will increase the vascular damage thus causing recurrent hemorrhages. In the alveoli and bronchi there are hyaline membranes and occasionally fragments of *Leptospira* or intact leptospiral organisms. In these cases the patient's condition will rapidly deteriorate, resulting in death.<sup>(13)</sup>

At postmortem, the heart frequently shows interstitial myocarditis with inflammation of the conducting system, coronary arteritis, and acute aortitis.<sup>(5,13,14)</sup> Approximately 90% of known cases are mild in nature and self-limited. When not treated with antibiotics, the spirochetemic phase generally will be followed by the immune phase.

## CLINICAL SYMPTOMS

In general, the clinical symptoms are extremely variable, ranging from mild subclinical symptoms with seroconversion, to very severe disease symptoms. Clinically there are two recognized leptospirosis syndromes, i.e. self-limited systemic disease in about 90% of infections, and an extremely fatal leptospirosis accompanied by renal failure, hepatic failure, and hemorrhagic pneumonitis.<sup>(4)</sup>

The course of illness is divided into two phases, i.e. the septicemic and immune phases. In the septicemic phase the bacteria are present in the circulation and subsequently spread to all organs; this is followed by the immune phase with grave symptoms (Figure 2).<sup>(13)</sup> In severe illness, the line separating both phases is indistinct. Occasionally the patients show symptoms only in the second phase.<sup>(4)</sup>

The disease commonly has a mean incubation period of 10 days (5-14 days). The septicemic phase is of acute onset, characterized

by an elevated temperature 38 to 40°C.<sup>(4)</sup> The clinical symptoms depend on the organs affected. The most prominent systemic findings are high fever with chills, sweating, nausea/vomiting, headache, joint pains, backache, wide-spread myalgia, particularly in the thigh and calf muscles, conjunctival suffusion, disturbances of vision, diarrhea, and skin rash.<sup>(2,3,7,15)</sup> Occasional signs are lymphadenopathy, splenomegaly, and hepatomegaly. The acute phase usually terminates after 5 to 7 days. If the leptospirosis has affected the lungs, there is coughing that may lead to hemoptysis, and dyspnea leading to respiratory failure, such that the patient requires respiratory-assist devices.<sup>(8)</sup> The patient frequently also shows jaundice as a result of *Leptospira* invasion of the liver. Severe illness is commonly accompanied by manifestations of the central nervous system, such as severe headache, photophobia, visual disturbances, lowering of consciousness and delirium. Aseptic meningitis occurs in 5-24% of patients. Ophthalmic findings include conjunctival suffusion, iritis, iridocyclitis, chorioretinitis, chorioiditis, photophobia, blurred vision, and ocular pain. The ophthalmic findings appear a few days up to 18 months after acute infection.<sup>(15)</sup> Gastrointestinal complaints comprise abdominal pain, decreased appetite, vomiting, diarrhea, hematemesis, leading to fatal gastrointestinal hemorrhage. Occasionally, there are symptoms of cholecystitis without biliary calculi, and pancreatitis.<sup>(16)</sup> There have also been reports of hemorrhages in cardiac muscle, causing disturbances of cardiac rhythm demonstrable on electrocardiographic examination, and resulting in sudden death.<sup>(11,14)</sup> Nevertheless, reports on deaths in the acute phase are rare.

The acute phase is followed by the immune phase, which commonly ends within 4-30 days. The disappearance of *Leptospira* from the blood and cerebrospinal fluid takes place concurrently with the appearance of IgM antibodies. Leptospire may be found in nearly all tissues

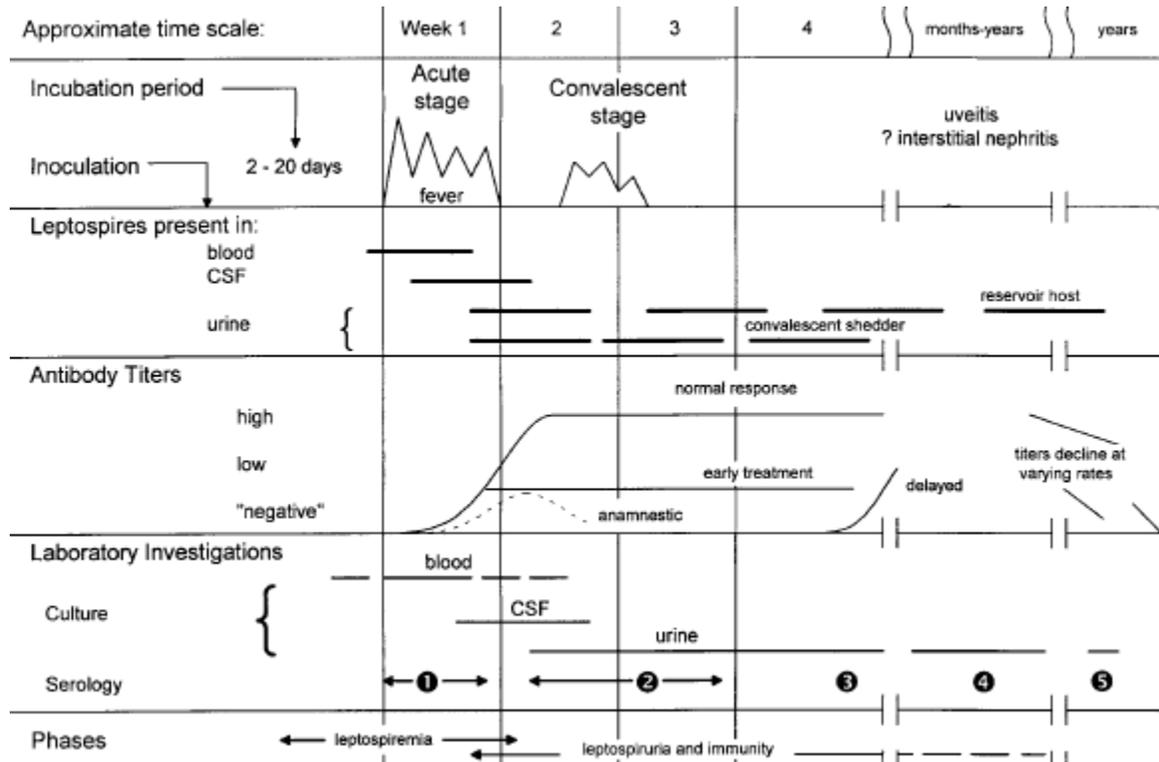


Figure 2. Course of illness in leptospirosis<sup>(13)</sup>

and organs, and are detectable in urine up to a number of weeks, depending on the severity of the illness. The following findings may still be encountered in the immune phase: jaundice, renal failure, cardiac arrhythmias, pulmonary disorders, aseptic meningitis, conjunctival suffusion (with or without hemorrhage), photophobia, ocular pain, adenopathy, and hepatosplenomegaly.<sup>(4,14)</sup>

Aseptic meningitis, either symptomatic or asymptomatic, is a characteristic sign of the immune phase, and is found in more than 80% of patients. In areas endemic for *Leptospira*, the majority of aseptic meningitis cases are due to infection with *Leptospira*. The patient complains of extremely severe frontal and temporal headaches, with or without lowering of consciousness. Occasionally the presence of grave neurological complications have been

reported, such as coma, transverse myelitis, or the Guillain-Barre syndrome.<sup>(4)</sup> The most severe form developing after the acute phase is Weil's disease, marked by hepatic and renal failure. In the acute phase occasionally a more severe illness develops, with fever of more than 40°C, followed by severe hepatic abnormalities, renal failure, cardiac arrhythmias, and shock appearing within an extremely short period of time, with no signs of improvement.<sup>(4,14)</sup> There is also acute renal failure marked by symptoms of uremia and oliguria appearing suddenly in the immune phase, and demonstrated by abnormal laboratory results. Frequent pulmonary signs are hemoptysis and dyspnea leading to respiratory failure. Radiologically, severe pulmonary abnormalities are predominantly found in the lower lobes, developing from small snowflake-like solid nodules into patchy alveolar infiltrates.

Extensive pulmonary consolidation is rarely encountered.<sup>(8,13,14)</sup> Heart failure is seldom found; however, electrocardiographic abnormalities are frequent. More than 50% of patients on cardiac monitoring experience cardiac arrhythmias, including atrial fibrillation, flutter and tachycardia, premature ventricular contractions, and ventricular tachycardia.<sup>(4,14)</sup>

## PROGNOSIS

There are many factors affecting the prognosis of patients with leptospirosis. In severe cases the mortality rate may reach 5-52%.<sup>(4,11)</sup> There are 5 factors determining the occurrence of death, i.e. dyspnea, oliguria, a leucocyte count exceeding 12,900/mL, repolarization abnormalities on electrocardiograph (ECG), and the presence of alveolar infiltrates on chest radiographs. The mortality rate is higher in adults than in children,<sup>(17)</sup> and is not associated with the infecting serovar,<sup>(18)</sup> while in general those succumbing are the elderly.

## LABORATORY INVESTIGATIONS

The results of peripheral blood examination in the acute phase are not specific, but indicate the presence of bacterial infection. The erythrocyte sedimentation rate (ESR) is generally raised, and there is thrombocytopenia and leucocytosis. *Leptospira* organisms are detectable in the blood and cerebrospinal fluid in the acute phase. *Leptospira* may also be found in the urine from the fifth to the seventh day after onset of symptoms. Urinalysis results show mild proteinuria and pyuria, with or without hematuria, and hyaline and granular casts.<sup>(4,12)</sup> Renal abnormalities are demonstrable by the hematological findings of elevated blood urea nitrogen levels, but not exceeding 100 mg/dl, and raised serum creatinine concentrations around 2 to 8 mg/dL. There is a reduced platelet count without signs of disseminated intravascular

coagulation (DIC); in contrast, signs of progressive renal disease are present.<sup>(4,14,18)</sup>

Cerebrospinal fluid examination in a severe immune phase will show increased cell counts, but not more than 500/mm<sup>3</sup>, consisting of lymphocytes. Protein is slightly increased, about 5-100 mg/mL, while glucose concentrations are within normal limits.<sup>(4)</sup> Patients with severe jaundice will show hepatic pathological findings disproportionate to the visible jaundice. The serum indirect bilirubin concentration is increased up to 80 mg/dL, with only a slight increase in the transaminases, i.e. alanine and aspartate aminotransferases. In contrast to viral hepatitis, the increase is rarely more than 200 µ/L. The jaundice will gradually disappear.<sup>(4,14)</sup>

## DIAGNOSIS

A diagnosis of leptospirosis solely on the basis of clinical findings is extremely difficult to establish, as there are many diseases with similar findings. Consequently, a definite diagnosis requires laboratory investigations. There are numerous laboratory methods suitable for diagnosis of human leptospirosis, such as (i) direct detection of *Leptospira* in specimens using dark-field microscopy or polymerase chain reaction (PCR), (ii) detection of *Leptospira* in specimens after prior culture, using dark-field microscopy or PCR, (iii) detection of antibodies using the microagglutination test (MAT), enzyme-linked immunosorbent assay (ELISA) and various other methods.<sup>(4,11,13)</sup>

### Direct method using dark-field microscopy

This requires expert and experienced laboratory personnel and yields low positive results (has low sensitivity). The specimens for examination are usually collected from tissues or fluids such as blood, urine, cerebrospinal fluid, or are cultured beforehand. Specimens most frequently yielding positive results are

blood specimens, particularly those collected in the early phases. *Leptospira* organisms are rarely found in the urine, as the leptospire are not resistant to acid environments. The collected specimens may also be cultured prior to examination. However, examination of microscopic specimens that must be cultured in advance is very time-consuming, because *Leptospira* grows very slowly in culture, requiring up to several weeks, such that this test cannot be used for rapid diagnosis.<sup>(11)</sup>

#### **Detection of *Leptospira* using PCR**

*Leptospira* may also be detected by using the PCR. The results obtained are of high sensitivity and specificity, as PCR is capable of detecting *Leptospira* organisms in numbers as low as 10.<sup>(19)</sup> Another advantage of this test is its capacity for detecting the disease in the earlier phases. In addition, PCR is able to detect leptospiral DNA in cerebrospinal and intraocular fluids, at a time when leptospiral antibodies have not yet entered the blood stream.<sup>(20)</sup> The disadvantages of this technique are its requirement for expert and experienced personnel, and the high price of the equipment, so that it is not always available in all laboratories, especially in developing countries.<sup>(11)</sup>

#### **Detection of *Leptospira* antibodies using MAT**

The diagnosis of leptospirosis is generally established based on detection of serum antibodies using the MAT method. However, this technique is extremely complicated, as it requires expert and experienced personnel, a large number of live serovars, and a knowledge of the serovars prevalent within the particular area. The results obtained frequently arrive too late and are incorrect. This technique requires live leptospire, thus posing extreme dangers to the laboratory personnel involved. Moreover, cross-reactions are frequent and the test is difficult to

standardize.<sup>(4,11)</sup> Although MAT has many weaknesses, it is still being used world-wide as a diagnostic standard for leptospirosis. Samples should be collected serially in duplicate, one sample in the acute phase and one in the convalescent phase. If the titer of the second sample is four-fold that of the first, then the test is considered positive. Occasionally, only one sample is collected.<sup>(11)</sup> Evaluation of the result of one sample only is more difficult, because it requires knowledge of the minimum titer considered positive. This minimum titer is different for each [geographic] area, depending on the seroepidemiological data for that area. Generally in areas with frequent epidemics, the minimum level of antibodies is higher than in non-epidemic areas.<sup>(13)</sup> In the United States an antibody titer of over 1:200 is already considered positive, whereas for tropical developing countries with a relatively high number of leptospirosis cases, a positive level of over 1:800 is recommended. The antibody titer in convalescent patients may reach 1:800 and this may commonly last up to 13 months, while a titer of 1:192 may last up to seven years after infection.<sup>(21)</sup>

#### **Detection of *Leptospira* using ELISA**

ELISA is a recent diagnostic technique capable of more specifically detecting *Leptospira* antibodies, comprising both IgM and IgG. IgM antibodies can be detected on day 2 to day 6 after onset of symptoms, with a sensitivity of 100% and a specificity of 94%, in comparison to MAT. However, in about 29% of patients IgM antibodies can be detected earlier. IgG antibodies are detectable on day 5 to day 8. IgM antibody responses occur in all patients, while IgG antibody responses occur in less than 90% of patients.<sup>(22)</sup> Based on above findings, the leptospire dipstick was developed. The examination of IgM using this technique does not require electricity for cooling equipment or refrigerators. This

technique is extremely easy to perform and the costs are low. The dipstick test uses a broadly reactive *Leptospira* antigen, fixed on a solid support. When dipped into serum containing IgM-antileptospiral antibodies, the anti-IgM-dye conjugate forms an antigen-antibody complex, thus giving rise to a colored band, which may subjectively be assigned a score from 1 to 4. The ratio of acute to convalescent serum titers in each case may be used for establishing the diagnosis.<sup>(23)</sup> Recently the lepto dipstick has been evaluated in 12 countries from 5 continents on 2,665 serum samples from 1,057 patients.<sup>(24)</sup> In the early phases of the disease, the lepto dipstick had a sensitivity of 86-35% and a specificity of 99-86%. The results of samples collected at a later time indicated that on average its sensitivity increased to 87%, from 60%. Based on these facts, it is suggested that the test be used particularly in countries with currently inadequate laboratory facilities.

### Differential diagnosis

*Leptospira* exhibits extremely variable clinical findings, so that it is difficult to differentiate from other diseases. Particularly in the tropics the incidence of diseases mimicking leptospirosis is very high, thus misdiagnoses are extremely frequent.<sup>(25)</sup> The climatic condition for the occurrence of epidemics of leptospirosis is identical to that for dengue fever epidemics, i.e. the rainy season. In the early stages both diseases are very difficult to differentiate, presenting with headache, elevated body temperature and myalgia.

Another disease to be differentiated from leptospirosis is hemorrhagic fever with renal syndrome (HFRS) caused by hantavirus, found in Asia and Europe accompanied by renal disorders. HFRS also exhibits acute fever, affects the kidneys and is occasionally accompanied by pulmonary disorders. The source of transmission of this disease is also the

rat, thus HFRS epidemics may occur concurrently with those of leptospirosis, i.e. in the rainy season.<sup>(26)</sup> Other hantaviruses, e.g. the South American Andes virus, also present syndromes similar to leptospirosis, such as pulmonary vascular abnormalities, shock, and the occurrence of the acute pulmonary failure syndrome, resulting in death. This infectious disease may also be of endemic character.<sup>(27)</sup> Furthermore, rickettsioses also have an epidemiological profile similar to leptospirosis.

### TREATMENT

Theoretically *Leptospira* is sensitive to antibiotics, and the antibiotic considered having adequate potency as antileptospiral drug is tetracycline. Tetracycline is capable of reducing the duration of fever and other symptoms, but it cannot prevent the occurrence of renal and hepatic abnormalities. Penicillin is more effective in reducing the duration of fever and other symptoms, especially if administered within the first four days after onset of symptoms. This antibiotic is capable of preventing hepatic and renal abnormalities, and also nuchal rigidity and hemorrhage, but it cannot reverse existing organ damage. In severe cases the administration of 1,500,000 – 2,000,000 units of intravenous penicillin every six hours is recommended. In children penicillin G may be given, 100,000 units/kg body weight/day for 5-7 days, which may reduce the duration of illness.

Doxycycline given in oral doses of 100 mg twice daily reduces the duration and severity of illness, and can be used in prophylaxis of *Leptospira* infection. However, at the time this study was undertaken, there were no severe cases of leptospirosis, thus using this antibiotic in severe cases is still of dubious merit.<sup>(24)</sup>

Ampicillin may also be given as an antileptospiral drug. The results of a study in children given 100 mg/kg body weight/day for

5-7 days indicate that this drug may reduce the duration of illness. However, extreme care is called for when administering antibiotics, because these drugs may induce the Jarisch-Herxheimer reaction, comprising acute fever, headaches accompanied by myalgia, and hypotension. The reaction commonly occurs 4-5 hours after administration of intravenous penicillin. The pathogenetic mechanism is still unclear, but the reaction is considered to be due to lysis of *Leptospira* organisms by the antibiotic, thus resulting in the release of toxins, which in turn cause induction of cytokines. However, occurrence of this reaction is extremely rare.<sup>(29)</sup>

In severe cases, in addition to administration of antibiotics, supportive therapy is indicated for dehydration, hypotension, hemorrhage, renal failure, pulmonary disorders, hepatic disorders, and disorders of the central nervous system. Administration of corticosteroids should be considered for prevention of chronic sequelae.<sup>(11)</sup>

## PREVENTION

Prevention of *Leptospira* infection comprises avoidance of high risk exposures, use of protective clothing, immunization, and chemoprophylaxis.<sup>(4)</sup> When working in a contaminated environment, e.g. farmers in wet soils at high risk of *Leptospira* infection, it is advisable to don protective wear, such as boots and rubber gloves. For short-term prevention of *Leptospira* infection, as when entering an area endemic for leptospirosis, use of chemoprophylactic drug is suggested. Administration of doxycycline 200 mg weekly for 4 weeks may prevent leptospiral infection.<sup>(29)</sup> Prophylaxis may be more beneficial if administered to communities in areas with epidemics (outbreaks).<sup>(30)</sup>

In developed countries such as Europe and the United States, immunization of humans for prevention of leptospirosis has not been widely

practised. Immunization with polyvalent vaccines has already been undertaken in Far Eastern countries, such as China and Japan, where the number of leptospirosis cases are relatively high, the majority of the populations being farmers. In France, a monovalent vaccine containing the icterohemorrhagiae serovar has been used in humans, while in Cuba a polyvalent vaccine has been developed for immunizing humans.<sup>(11)</sup>

Long-term prevention of human leptospirosis by vaccination is apparently not effective. This is due to the wide variation in lipopolysaccharides of the respective serovars, such that the antibodies produced are also extremely variable. Each area has a specific range of prevalent serovars, differing from other areas. This represents a challenge to the development of human vaccines capable of preventing all serovars in a given area.<sup>(11)</sup>

## CONCLUSIONS

Leptospirosis has extremely variable symptoms such that it is difficult to differentiate from other diseases with similar symptoms. This disease is extremely difficult to diagnose, as the techniques for examination are very complicated, require expert and experienced personnel, and are of rather low sensitivity, with the consequence that occurrences of this disease are notified only rarely. Leptospirosis is treated by administration of antibiotics, such as penicillin, tetracycline, ampicillin, and amoxicillin. Prevention by immunization is as yet unsatisfactory, thus the currently best method is by avoiding exposure to contaminated environments.

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